periodic lamellipodial protrusion retractions cycles that require a rigid substrate and the development of adhesions[1]. Moreover, there is evidence that rigidity is detected locally in the region near the cell edge[2]. The motor protein myosin II powers cell retractions, which are simultaneous with rearward flow of the actin filament network1. However, the pattern and mechanism of forces exerted by cells to test matrix rigidity have not been characterized. using high-resolution force sensing pillars, we show here that the primary forces powering periodic edge retraction come from local contraction units, which exert inward forces near the cell edge, correlated with outward forces 2-3 micrometers rearwards from the edge. Pillar displacement correlates with the local concentrations of active myosin II and the actin crosslinking protein alpha-actinin, but not with local levels of other common actin binding proteins. The contractile units therefore display similarity to striated muscle and stress fiber units, which are ~1-3 micrometers long, rely on myosin II for contractility, and contain alpha-actinin as a major component of the complex that anchors actin filaments. Thus, our results indicate that local contractile units, with sarcomere-like organization, are responsible for periodic cell edge retractions and rigidity sensing. References: [1] G. Giannone, B. Dubin-Thaler, M. Sheetz et al. (2007) Cell 128, 561-575. [2] S. Ghassemi, G. Meacci, J. Hone et al. (2012) Proc. Natl. Acad. Sci. USA, 109, 5328-5333

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Myofibril Acquisition from Primary Rat Cardiomyocyte Culture for Mechanical Studies

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Introduction: Myofibril based mechanical studies allow targeted evaluation of sarcomeric protein function. Here we describe a novel method of obtaining myofibrils from primary cardiomyocyte culture.

Methods: Adult rat ventricular myocytes (ARVMs) were obtained by enzymatic digestion and maintained in serum free environment for 72 hours. ARVMs were homogenized in relaxing solution (pCa 9.0) with 20% sucrose, and myofibril suspension was made. Myofibrils, mounted on a force recording apparatus, were Ca^{2+}-activated and fully relaxed by fast solution switching at 15°C and 2.1 μm initial sarcomere length.

Results from ARVM myofibrils were compared to those myofibrils obtained from ventricular tissue with traditional skinning method (0.05% Triton-X100). Results: At maximal Ca^{2+}-activation (pCa 4.5) myofibril mechanical parameters from ARVMs were 8 mN/mm2 (resting tension), 156.4 mN/mm2 (maximal active tension, Po), 5.4 s-1 (rate of force activation, kACT), 5.2 s-1 (rate of force redevelopment following release-restretch, kTR), 41.6 ms (linear relaxation duration), and 13.6 s-1 (exponential relaxation rate). The parameters of the myofibrils isolated from the ventricular tissue were not significantly different. Degree of run-down (decrease in Po and kACT between subsequent activation-relaxation cycles) was evaluated. Between first and third activations, we noted the following % changes in myofibrils from ARVM cultures: −23.6% (Po), −29.6% (kACT). The magnitude of run-down was not statistically different between myofibrils from ARVMs versus the traditional Triton skinned samples. Finally, pCa50 from Triton skinned, ARVM culture day 0, and ARVM culture day 3 myofibrils were 5.76, 5.74, and 5.71, respectively.

Conclusion: Myofibrils obtained from ARVMs is a viable method to study myofibril mechanics. This culture based approach to obtaining myofibrils will allow pharmacological and genetic manipulation of the cardiomyocytes to correlate biochemical and biophysical properties.

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Phosphorylation alters the Mechanical Properties of Keratin Filaments in Living Cells

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Among the three types of intracellular filament types, only the intermediate filaments (IFs) are able to withstand high loads of tensile stress. Despite the pathophysiological relevance of this mechanical support function in living cells, our current knowledge about the mechanical properties of IF is almost exclusively based on in vitro experiments with isolated IF. In our study we stretched living cells uniaxially by 80% for 30s to determine the extension of keratin filaments (the IF of epithelia) and we investigated how the filaments behave after stretch release. Moreover we used keratin mutants that mimic or abrogate phosphorylation of keratin 8-serine431 and keratin 18-serine52 to further investigate whether phosphorylation affects the mechanical properties of keratin filaments. When cells are stretched and released the keratin filaments buckle, discernible by tortuous keratin filaments. Our experiments show that this tortuousness is not simply caused by a plastic overextension of keratin filaments but requires an intact actin-myosin system. Probably actin/myosin provide a scaffold for cross linking proteins (e.g. plectin) to anchor the keratin filaments in their extended state during stretch. When the stretch is released, the scaffold is pushed together and the cross linkers buckle the keratin filaments. Interestingly, no filament tortuousness was found when cells were transfected with keratin mutants where phosphorylation of keratin18-serine52 was abrogated. We propose that dephosphorylated keratin18-serine52 strengthens the recoil force of keratin filaments and enables keratin to overpower the buckling force exerted by the compressed scaffold. Alternatively, the keratin dephosphorylation might weaken its binding to cross linker proteins, permitting a 'frictionless' slipping back of the filaments after stretch release. We found that keratin18-serine52 is predominantly phosphorylated in the cell periphery, which points at a particular role of the peripheral keratin network in this regard.

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Characterization of the Vimentin Intermediate Filament Network on Substrates of Varying Stiffness

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Cells, in vivo, generally live in environments that are much softer (~kPa stiffness) than tissue culture plastic or glass on which they are usually studied (~GPa stiffness). While the response of the microtubule and the actin cytoskeletal networks to substrate stiffness has been previously studied, the response of the vimentin intermediate filament network to changing substrate stiffness is not known. When cells are grown on different stiffness polyacrylamide gels, there is no change in the total amount of vimentin protein. However, there is a significant change in the amount of vimentin protein that can be extracted by Triton-X 100 when the cells are grown on substrates of physiological stiffness. In human mesenchymal stem cells (hMSCs) cultured on glass, less than 5% of the vimentin is in the soluble pool. On substrates of physiological stiffness, the amount of soluble vimentin responds in a biphasic manner. The amount of soluble vimentin increases as stiffness decreases to a peak of about 65% soluble pool in cells cultured on a 5 kPa substrate. The amount of soluble vimentin then decreases again as substrate stiffness decreases to 0.2 kPa. This phenomenon appears dependent on contractility, as on stiff gels treated with blebbistatin or cytochalasin D the soluble vimentin pool increases and there is no change in soluble vimentin on 5 kPa gels. In addition, cells grown in a confluent monolayer on a 5 kPa gel show a significant decrease in the soluble pool. These observations can help to elucidate the function of the vimentin network, and suggest that in vivo cells maintain a much larger pool of dynamic vimentin than is seen under standard tissue culture conditions.

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Cell Visco-Elasticity Measured with AFM and Vertical Optical Trapping at Sub-Micrometer Deformations

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Cell mechanical properties are routinely quantified by indenting the cell with a probe of an AFM. Because the resulting deformations are relatively large (~μm), the mechanical contribution of the different parts of the cell is expected to change during the indentation. This results in a non-linear response of the cell as a whole, with both elastic and viscous properties.

We used a combined AFM and vertical optical trapping approach to deform single fibroblasts with a micrometer-sized bead at forces from 10 pN to 1 nN. At low indentations (0.2 μm) the cellular response is mainly elastic; the modulus of 100 Pa is largely determined by the actin cortex. At higher indentations, viscous effects increase the apparent elastic modulus. This viscous contribution followed a weak power-law with an exponent of up to 0.31 (at 1 μm). Optical trapping has the benefit of a lower force noise than AFM and gives consistent results for the cell's visco-elasticity. The combination of both techniques allows the investigation of single cells at a wide range of length- and time-scales which enables the separation of their viscous and elastic components.

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Laser-Generated GHz Acoustic Waves Reveal a Universal Nuclear Stiffness Probed during Cell Differentiation

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